## A. curcumene (0.43%). essential oils (eo) are secondary

Business, Accounting



A. Composition of turmeric essential oil The active compounds of the essential oilextracted from Curcuma longa L. through the hydrodistillation method were determined using Gas Chromatography/MassSpectrometer (GCMS) analysis and the results are presented in Table 2. From Table 2, GC-MS analysis on the turmericoil extracted from turmeric rhizome identified that there are 5 predominant compounds, accounting for 71% of total peak area. The active compounds are turmerone (35. 46%), cumene (20. 61%), Arturmerone (13.

82%), cymene (0. 90%) and curcumene(0. 43%). Essential oils (EO) are secondary metabolites in plant (Ciocan andBara, 2007) and typically have compounds with isoprenestructures such as terpenes with the general chemical structure C10H16like diterpenes (C20), triterpenes (C30), tetraterpenes(C40), hemiterpenes (C5) and sesquiterpenes (C15). When the compounds contain an additional oxygen, it is known as terpenoids. Themajor groups of the active compounds are classified as terpenes (cumene, cymeneand curcumene) and terpenoids (turmerone and Arturmerone). Similar findingshave been observed in a study by Liju et al.

(2016) where they found that the major compounds of essential oil from turmeric oil are ar-turmerone, curlone and ar-curcumene with chemical constituents of 61. 79%, 12. 48% and 6. 11%, respectively. The antimicrobial properties of the essential oil fromplant extracts are resulted from the synergy of its active compounds (Avanço et al., 2017).

Table 2 shows that turmerone is the mostconcentrated major functional group in the turmeric oil used as the antimicrobial agent in this study. Similar

behaviour was found in several studies on the extraction of essential oil of turmeric from different origins established that the predominant compound is turmerone (Avanço et al., 2017; Gupta et al., 2015; Liju et al., 2016; Singh et al.

, 2011; Stanojevi? et al., 2015). Thus, it was expected that the antimicrobialactivity of the turmeric oil is attributed to the high concentration ofturmerone (35. 46%). This hypothesis is supported by other studies on the antimicrobialactivity of terpenoid group on specific pathogens. A study by Cabral et al. (2013) on terpenoid identified that these compounds provideantimicrobial properties against bacteria, protozoa, fungi and also virus.

A reviewstudy done by Chandra et al. (2017) on the mechanism of antimicrobial action ofactive compounds classified as terpenoids stated that terpenoids from the barkof Acacia nilotica have antimicrobialactivity against S. viridans, S. aureus, E. coli, B.

subtilis and Shigella sonneiwhile terpenoids from Cymbopogon citratushave moderate activity against C. albicansand low activity against P. aeruginosa, E. coli, S.

aureus and T. mentagrophy. Similar findings have been observed in studies by Knobloch et al. (1986) and Sikkema (1994) where the authors stated that EO containingterpene and terpenoid compounds have the ability to react with lipids on themicrobes cell membrane and cause membrane permeability which leads to the deathof the cell. B.

Shelflife of breadThe shelf life of bread was studied by wrappingthe bread in both the commercial packaging and the antimicrobial film coated onKraft paper for food packaging as shown in Table 3. Referring to Table 3 allthe films that were incorporated with varying concentrations of turmeric oil at0. 5, 15 and 30 ? L/L managed to extend the shelf life of bread by 4, 8 and 7days respectively after the expiration date compared to commercial packagingwhich extended the shelf life by only 2 days. Similar findings were found by Nilda (2014) where the author reported that newlydeveloped edible films incorporated with clove and oregano oils preserved breadlonger than the commercial additive, calcium propionate. After 10 days, Nilda establishedthat bread slice with commercial preservative lost its effectiveness but ediblefilms containing small droplets of the oils continued to inhibit the growth of themould (Nilda, 2014). C. Analysis of variance (ANOVA) by response surface methodology (RSM)The significance and fitness of theindependent factors evaluated in RSM of the biopolymer films coated on Kraftpaper were determined from the antimicrobial agent migration rate, antimicrobial activity and weight loss in biodegradation test.

The results are presented in Table 4. As for the statistical analysis, the model of coefficients was calculated by using multiple linear regressions (Myers and Montgomery, 2002) and validated using analysis of variance (ANOVA). Results of the variance analysis (F-values) for each dependent variable and the respective coefficients of determinations (R2) for second-order response model are also presented in Table 4. The effects of biopolymer film thickness containing different concentrations of turmeric oil on the dependent variables are represented using response surface plot as shown in Figure 1.

1) Antimicrobialagent releaseTable 4 shows the analysis of the linear (A, B), quadratic (A2, B2) and interaction terms (AB) effects on the efficacy of the biopolymer film to release antimicrobial agent. From the results obtained, the model F-value of 18. 62 indicated that the quadratic model for antimicrobial agent release is a significant model with only 0.

01% chancethat a "Model F-Value" this large could occur due to noise. The results for alllinear (A, B), quadratic (A2) and interaction (AB) coefficient havesignificant (P < 0. 05) effect on antimicrobial agent release. Figure 1a.) shows the response surface plotsof biopolymer film thickness and concentration of turmeric oil on antimicrobial agent release. Theresults indicate that the antimicrobial agent release increases when thebiopolymer film thickness increases until it reaches a maximum value of approximately1.

50 mm. Past the maximum value, the release decreases at higher thickness. Increasing the biopolymer film thickness will increase the availability of antimicrobialagents in the food packaging which contributes to higher antimicrobial agentrelease. Theoretically, antimicrobial agent release is the migration of theagent down a concentration gradient, usually from packaging to food simulant. However, a further increase in film thickness exceeding 1. 50 mm will increase the netdistance for the antimicrobial agent to diffuse from the biopolymer film tofood surface. Hence, the antimicrobial agent located too far from thefood-simulant may not be able to migrate into the food matrix as the averagemigration distance is exceeded and migration only occurs in the packagingmatrix.

A study by Simon (2008) stated that if the packaging is not incontact with the food, the active compounds motion will occur and distribute evenly in the polymer matrix while the concentration remains unchanged. Although, at the minimum value approximately 15? L/L, it shows a slight increase over the whole explored concentration range. This contradicts the trend of the effect of concentration of turmeric oil. In contrast, a study by Simon (2008) stated that migration of active compounds from base packaging to food will progressively take place until it reaches equilibrium in concentration between the packaging and food. The decrease inantimicrobial agent release as the concentration was increased to 15? L/L can beattributed to the stronger interaction between the antimicrobial agents with the biopolymer film compared to the interaction between the antimicrobial agentand foodsimulant.

However, further research would be needed to reassess that. Thus, selecting the right amount ofantimicrobial agent and film thickness are vital to inhibit food spoilage activityby microorganisms in order to increase the shelf life of desired food products significantly. Besides, if the targeted microbes have very short lag periods such as fungus, it is ineffective to use thick film which will release the antimicrobial compound very slowly over a period of time or very thin film as theantimicrobial compound might be released too rapidly and thus defeats its mainpurpose which is to extend the shelf life of the food products (Malhotra et al., 2016). 2) Inhibitionzone of A. nigerOnly the concentration of C. longa L.

affects the inhibition zone of A. niger significantly as shown in Table 4 with B (P < 0.05).

Themodel F-value of 3. 85 indicates that the quadratic model for inhibition zone of A. niger is a significant model withouth 3. 30% chance that a "Model F-Value" this large could occur due tonoise. Referring to Figure 1, the clear zone of A. niger growth was observed for all different concentrations of turmeric oil and this indicated that the antimicrobial film successfully inhibited the growth of A. niger (Figure 2).

Increasing the concentration of turmeric oildecreased the inhibition zone of A. nigergrowth up to 15? L/L of turmeric oil with the largest inhibition zone wasobserved on film with concentration 0. 5? L/L of turmeric oil. The resultsobtained show similar behaviour as previous studies on antimicrobial agentrelease.

The study by Singh et al. (2012) on essential oil of turmeric rhizome showedthat turmeric oil inhibited the growth of A. nigerwith a minimum inhibitory concentration of 6. 7 ? L/L. Meanwhile, in this study, the film itself can inhibit the growth of microbes but by adding the turmeric oil, the antimicrobial activity of the film is enhanced. Thus, it can be stated thatin this study the minimum inhibitory concentration of turmeric oil added in thefilm is 0 ? L/L.

The inhibition activity exhibited by the film itself may be attributed to the dense structure of the film and presence of hydroxyl compound in the film which do not allow the growth of A. niger. Delaquis et al. (2002) reported

that the antimicrobial properties of alcohol compound increase with molecular weight, in which both cassava starchand carboxymethylcellulose have high molecular weight with the presence of alcohol.

Inhibition zone of A. Nigergrowth increased as the concentration of turmeric oil was also furtherincreased to 30? L/L. At very high concentration of turmeric oil in the film, thepresence of hydroxyl compound acting as antimicrobial agent also increased andthis led to the formation of 'bound' hydroxyl compound with the biopolymer baseinstead of 'free' hydroxyl compound to react with the lipids on the cellmembrane. It is desired for the 'free' hydroxyl compound to react with the cellmembrane lipids so that the membrane becomes permeable and subsequently killthe cell (Sikkema, 1994).

3) Biodegradationin multipurpose compostThe model F-value 9. 51 as shown in Table 4indicates a significant model. There is only a 0. 15% chance that a "ModelF-Value" this large could occur due to noise.

Value of P> F less than 0. 05indicates that the model is significant and the study shows that A (thickness) is a significant model term. This means that the response is significantly affected by coating thickness instead of concentration of turmeric oil. Referring to Figure 1c.), the sampleweight loss during the biodegradation test was directly proportional to coating thickness and increased as turmeric oil concentration was increased from 0 to 15 ? L/L.

This may be attributed to the presence of polar groups in bothbiopolymer and essential oil which allowed the samples to form a hydrogen bondwith water and this subsequently accelerated the decomposition of the samples. This finding is supported by Souza et al. (2013) where the authors identified that the incorporation of cinnamon essential oil decreases the water barrier properties of biodegradable films. This is due to the introduction of oil which reduces the molecular interaction between polymeric chains and allows water uptake on the film. However, the samples weight loss was decreased when turmeric oil concentration exceeded 15 ? L/L withincreasing thickness. This is because the presence of turmeric oil which is hydrophobic in nature at a concentration higher than 15? L/L may change the hydrophilic properties surface of the film, hence reducing the water uptake and consequently decreasing the biodegradation rate of the samples.